CONCISE COMMUNICATION

Central Nervous System Activation of the Indoleamine-2,3-Dioxygenase Pathway in Human T Cell Lymphotropic Virus Type I–Associated Myelopathy/Tropical Spastic Paraparesis

Elizabeth M. Maloney,[1] Owen St. Claire Morgan,[2] Bernard Widner,[3] Ernst R. Werner,[3] and Dietmar Fuchs[3]

[1]Viral Epidemiology Branch, National Cancer Institute, Bethesda, Maryland; [2]Department of Medicine, University of the West Indies, Kingston, Jamaica; [3]Institute of Medical Chemistry and Biochemistry, University of Innsbruck and Ludwig Boltzmann Institute of AIDS-Research, Innsbruck, Austria

Human T cell lymphotropic virus type I (HTLV-I) is associated with a chronic neurologic disease called HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP). The potential mechanisms of HAM/TSP pathogenesis were assessed by examination of 2 pathways initiated by interferon-γ, a predominant cytokine in HAM/TSP. Jamaican HAM/TSP patients ($n = 17$) were compared with patients with other neurologic diseases (ONDs; $n = 13$) with respect to cerebrospinal fluid levels of the following: neopterin; nitrite plus nitrate, a stable indicator of nitric oxide; and tryptophan and kynurenine, metabolites of the indoleamine-2,3-dioxygenase (IDO) pathway. HAM/TSP patients had significantly elevated levels of neopterin ($P = .003$) and kynurenine ($P = .05$) and a significantly decreased level of tryptophan ($P = .003$), compared with patients with ONDs. These results support immune activation within the central nervous system and activation of the IDO pathway. Thus, activation of the IDO pathway may play a role in HAM/TSP.

Human T cell lymphotropic virus type I (HTLV-I) infection is associated with a chronic demyelinating neurologic disease called HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP). HAM/TSP is characterized by muscle weakness of the legs, hyperreflexia, clonus, extensor plantar responses, bladder dysfunction, impotence, and mild sensory disturbance. HAM/TSP patients have a mean age of 40 years and derive from geographic areas of HTLV-I endemicity, which include the Caribbean basin, southwestern Japan, parts of sub-Saharan Africa and South America, the Pacific Melanesian islands, and Papua New Guinea [1].

Immunologically, HAM/TSP is associated with a heightened immune response, as evidenced by the significantly increased spontaneous proliferation of lymphocytes, which include HTLV-I–infected T cells and cytotoxic T cells targeting infected cells [2]. Additionally, HAM/TSP is associated with activated T cell and T cell–induced expression of several inflammatory cytokines in peripheral blood and spinal cord lesions: interferon-γ (IFN-γ), tumor necrosis factor–α (TNF-α), and interleukin-1β (IL-1β) [3]. One theory of HAM/TSP pathogenesis purports that inflammatory cytokines produced by T cell–stimulated macrophages and glial cells may play a role in central nervous system (CNS) tissue damage in HAM/TSP [4]. We sought to examine CNS markers of 2 potentially pathogenic, IFN-γ–associated pathways in HAM/TSP disease.

One pathway involves the IFN-γ–stimulated macrophage production of nitric oxide (NO) during the conversion of L-arginine to L-citrulline, a process catalyzed by a variety of NO synthases [5]. NO is produced constitutively by neurons and endothelial cells so that they may act as neurotransmitters and vasodilators, respectively; NO production is induced by macrophages, microglia cells, and astrocytes, and it acts as a mediator of the immune system in its attack on pathogens [6]. While physiologic levels are healthy, excessive NO production can be neurotoxic. Several neurodegenerative disorders—including stroke, human immunodeficiency virus dementia, and Parkinson's disease, as well as cerebral malaria—have been associated with excessive production of inducible NO in *in vitro* studies [5]. We sought to determine whether NO was increased in HAM/TSP.

The second pathway involves IFN-γ–stimulated macrophage activation of the enzyme indoleamine-2,3-dioxygenase (IDO) and results in the degradation of tryptophan to kynurenine [6].

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Reprints or correspondence: Dr. Elizabeth Maloney, Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., MSC 7248, Rockville, MD 20822 (maloneyb@epndce.nci.nih.gov).

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Table 1. Cerebral spinal fluid biomarker measurements among patients with human T cell lymphotropic virus type 1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and patients with other neurologic diseases (ONDs).

Biomarker	Patients with HAM/TSP (n = 17)	Patients with ONDs (n = 13)	P
Nitrite plus nitrate, μmol/L			
Median	15.9	19.6	.66
Range	12.2–136.8	8.1–58.4	
Tryptophan, μmol/L			
Median	0.7	1.6	.0003
Range	0.1–1.6	0.6–2.5	
Kynurenine, μmol/L			
Median	0.2	0.1	.05
Range	0.1–3.0	0.1–0.7	
Kynurenine : tryptophan ratio			
Median	0.3	0.1	
Range	0.1–5.0	0.0–0.6	.001
Neopterin, nmol/L			
Median	28.0	3.4	.0003
Range	2.8–57.0	0.0–26.0	

NOTE. P values were obtained by using the Wilcoxon rank sum test.

Tryptophan is highly correlated with serotonin, an important neurotransmitter; thus, decreased tryptophan portends decreased serotonin [7]. It is possible that tryptophan plays a role in the neurologic symptoms associated with HAM/TSP.

Neopterin, a pterin derivative, is a commonly used marker of immune activation [8]. Like NO and kynurenine, it is produced in response to IFN-γ secretion and is elevated in association with many viral infections, intracellular bacterial infections, neoplastic conditions, and autoimmune diseases [8]. To assess general CNS immune activation, as well as specific pathways of immune activity, we measured neopterin; nitrite and nitrate, as an indicator of NO level; and tryptophan and kynurenine in patients with HAM/TSP and in patients with other neurologic diseases (ONDs).

Materials and Methods

Study participants. Study participants included 21 patients with HAM/TSP who were diagnosed between 1992 and 1996 at the neurology clinic at the University of the West Indies (Kingston, Jamaica) by one of us (O.S.C.M.). The controls included 18 patients who had visited the same clinic between 1990 and 1994. Three of the HAM/TSP patients who were HTLV-I seronegative and 1 OND patient who was HTLV-I seropositive were excluded from this analysis. Additionally, 1 HAM/TSP and 4 OND patients had an insufficient volume of cerebrospinal fluid (CSF) specimen for NO evaluation. The final sample size included 17 HAM/TSP patients and 13 OND patients.

Among HAM/TSP patients, onset of symptoms preceded diagnosis by a median of 1 year (range, 6 months–3 years). Diagnoses among controls included spinal cord compression/disk disorders (n = 3), benign intracranial hypertension (n = 2), motor neuron disease (n = 1), syringomyelia (n = 1), nerve schwannomas (n = 1), spinal stenosis (n = 1), meningitis (n = 2), amyotrophic lateral sclerosis (n = 1), and B_{12} deficiency (n = 1). CSF specimens were obtained at the time of diagnosis and were stored in a liquid-nitrogen freezer until withdrawal for analysis. Information on age, sex, and duration of symptoms (for HAM/TSP patients) was obtained at the time of diagnosis.

Biomarker measurement. NO decomposes in vivo to the stable products nitrite and nitrate. Nitrite and nitrate were used as markers of NO and were determined using the method of Green et al. [9], with modifications as detailed by Zangerle et al. [10]. Tryptophan and kynurenine levels were quantified using high-pressure liquid chromatography [11]. Neopterin was measured using a commercial RIA (Neopterin RIAcid; BRAHMS Diagnostica, Berlin).

Statistical methods. The Wilcoxon rank sum test was used to compare the study groups with respect to median ages, and the χ^2 statistic was used to compare the proportion of men in each group. The Wilcoxon test was used in a univariate analysis of median levels of biomarkers between HAM/TSP and OND patients. Spearman's correlation coefficient (r) was computed to examine a linear association between biomarker measurements and duration of symptoms among HAM/TSP patients. Separate logistic regression models were used to examine the association between HAM/TSP and biomarkers (dichotomized at the median value detected among OND patients), adjusted for potential confounders.

Results

HAM/TSP patients had a median age of 51 years, which was similar to the median age of 48 years recorded among controls (P = .66). However, female sex predominated among HAM/TSP patients (75.0%) compared with controls (42.9%); this difference approached statistical significance (P = .07). A comparison of median biomarker levels between HAM/TSP and OND patients is shown in table 1. The median CSF nitrite plus nitrate level for HAM/TSP patients was not significantly different from that of OND patients (P = .66), which indicated that overall NO production in these 2 groups was similar. Nitrite and nitrate levels were not correlated with duration of symptoms in HAM/TSP patients (r = .15, P = .58). Median CSF kynurenine and neopterin levels were significantly higher among HAM/TSP patients than among OND patients (P = .05 and .0003, respectively). By contrast, the median tryptophan level was significantly lower among HAM/TSP patients than among OND patients (P = .0003). As a result, HAM/TSP patients had a significantly higher median kynurenine : tryptophan ratio than did OND patients (P = .001). This ratio was more highly correlated with neopterin among HAM/TSP patients than among OND patients (r = .44, P = .08 and r = .24, P = .41, respectively). Kynurenine, tryptophan, the kynurenine : tryptophan ratio, and neopterin were not correlated with duration of symptoms (r = −.20, P = .46 [kynurenine]; r = .15, P = .59 [tryptophan]; r = −.02, P = .95 [kynurenine : tryptophan ratio]; and r = .32, P = .24 [neopterin]). Additionally, none of the biomarker measurements differed significantly by sex. Thus, adjustment for these 2 variables did not alter the results.

Discussion

The HAM/TSP patients in this study had a median age similar to that reported in other studies [1]. The predominance of female patients in this study group is typical of this disease, as risk of HAM/TSP is associated with sexual behavior, and male-to-female HTLV-I transmission is more efficient than the reverse [1].

Our HAM/TSP patients all had a relatively short duration of disease symptoms (<4.5 years). Short duration of disease (<4.5 years) is characterized pathologically by "active chronic" CNS lesions, featuring marked inflammatory changes; an equitable distribution of $CD4^+$ and $CD8^+$ cells and macrophages; increased expression of IL-1β, TNF-α, and IFN-γ; and frequent distribution of cytotoxic T cells and NK cells, immune system cells that target intracellular pathogens [3]. The significantly increased neopterin level among these patients is consistent with results obtained from Japanese HAM/TSP patients and indicates immune activation in the CNS [12].

In comparison with OND patients, HAM/TSP patients had a decreased CSF level of tryptophan, an elevated CSF level of kynurenine, and a significantly higher kynurenine : tryptophan ratio, demonstrating that CNS IDO activity is significantly increased in HAM/TSP patients [6]. These results agree with those of a prior report of serum biomarkers in Jamaican HAM/TSP patients, who were compared with groups of HTLV-I–seropositive and –seronegative controls [13]. The activated IDO pathway is an immunoregulatory mechanism that arrests cell-cycle progression at a tryptophan-sensitive checkpoint in the G1 phase of cell growth [14]. In the absence of tryptophan, commitment to cell division can be reversed, which, in turn, leads to cell-cycle arrest; provision of both tryptophan and mitogenic stimulation of the T cell receptor can overcome the arrested state and result in T cell division [14]. The HTLV-I tax protein has known mitogenic activity and is capable of inducing T cell transformation via the alteration of the cellular proteins that are responsible for cell-cycle suppression (p53 and p21) [15]. Thus, although tryptophan depletion may provide a mechanism to suppress the growth of HTLV-I–infected cells, the tax protein that is expressed in $\geqslant$90% of HAM/TSP patients may override this effect. In fact, the phenomenon of spontaneous lymphocytic proliferation, a hallmark of HAM/TSP, has been demonstrated in peripheral blood and may occur in the CNS, suggesting that degradation of tryptophan does not effectively suppress T cell proliferation in HAM/TSP patients [4]. Alternatively, a decrease in serotonin, an important neurotransmitter that correlates with decreased tryptophan, may be an inadvertent by-product of IDO activation [7]. Lower serotonin levels resulting from tryptophan metabolism by IFN-γ–induced IDO activity were suggested to be a mechanism in AIDS dementia [7]. Thus, HAM/TSP symptoms may result from activation of the IDO pathway.

HAM/TSP and OND patients had similar levels of nitrite plus nitrate, indicating that NO is not associated with HAM/TSP. The lack of a difference between patient groups may be due to sampling error. However, these results could also represent a true lack of relationship between HAM/TSP and NO at the time of diagnosis. Alternatively, activated macrophages alone may be insufficient for the overproduction that could potentially result in myelin damage; microglial and astrocyte production may be required as well [5]. Finally, if we link the findings on NO with the IDO metabolites, inhibition of inducible NO may result from IDO-dependent tryptophan degradation, which consumes oxygen radicals [16].

In summary, HAM/TSP was associated with immune activation of the IDO pathway within the CNS. Thus, decreased tryptophan may be an important contributor to HAM/TSP pathogenesis via its correlation with serotonin, an important neurotransmitter. NO was not associated with HAM/TSP in this study. Patient specimens were obtained at the time of diagnosis, which followed the onset of symptoms (of variable duration); thus, we cannot exclude an early effect of NO on the pathogenesis of HAM/TSP.

The HAM/TSP patients were all HTLV-I seropositive, whereas OND patients were HTLV-I seronegative; thus, it is uncertain whether the relationships discerned in this analysis reflect HTLV-I status or disease status. However, a prior study of HTLV-I carriers and HTLV-I–seronegative individuals showed no association between HTLV-I serostatus and any of these markers in serum [13], which suggests that HAM/TSP disease rather than HTLV-I infection is associated with changes in biomarker levels. We recommend further study of the association between tryptophan and serotonin in HAM/TSP patients.

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